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(54) Title: COMPOUNDS WITH A SULFAMATE GROUP		
(57) Abstract A sulphamate compound is described. The compound is suitable for use as an inhibitor of both oestrone sulphatase activity and aromatase activity. A preferred compound has general formula (II) wherein F represents a phenolic ring structure (the first ring structure), J represents the third ring structure, I represents a phenolic ring structure (the second ring structure), G is an optional double bond, H is a link joining the second ring structure to the third ring structure, and Y represents a suitable second group; wherein any one of ring structures F, J and I has bound thereto a sulphamate group.		

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COMPOUNDS WITH A SULFAMATE GROUP

The present invention relates to a compound.

5 In particular the present invention relates to a pharmaceutical composition comprising the compound.

Breast and endometrial cancers are major causes of death in Western women. In particular, tumours in endocrine-dependent tissues, such as the breast and
10 endometrium, occur most frequently in postmenopausal women at a time when the ovaries have ceased their production of oestrogens.

Evidence suggests that oestrogens are the major mitogens involved in stimulating and promoting the growth of tumours in endocrine-dependent tissues, such as the breast and endometrium²¹. Although plasma oestrogen concentrations are similar in women
15 with or without breast cancer, breast tumour oestrone and oestradiol levels are significantly higher than in normal breast tissue or blood. In addition, in postmenopausal women oestrogens continue to be produced by extraglandular production in adipose tissue but also in normal and malignant breast tissues²².

20 Figures 1 and 2 are schematic diagrams showing some of the enzymes involved in the *in situ* synthesis of oestrone from oestrone sulphate, oestradiol and androstenedione.

In Figure 2, which schematically shows the origin of oestrogenic steroids in
25 postmenopausal women, "ER" denotes Oestrogen Receptor, "DHA/-S" denotes Dehydroepiandrosterone/-Sulphate, "Adiol" denotes Androstenediol. "E1-STs" denotes Oestrone Sulphatase, "DHA-STs" denotes DHA-sulphatase, "Adiol-STs" denotes Adiol Sulphatase, and "17B-HSD" denotes Oestradiol 17B-hydroxysteroid dehydrogenase.

30 As can be seen, the main two enzymes that are involved in the peripheral synthesis of oestrogens are the aromatase enzyme and the enzyme oestrone sulphatase.

In short, the aromatase enzyme converts androstenedione, which is secreted in large amounts by the adrenal cortex, to oestrone. Recent reports have suggested that some flavones could inhibit aromatase activity^{35,36}.

5 Much of the oestrone so formed, however, is converted to oestrone sulphate (E1S) and there is now a considerable body of evidence showing that E1S in plasma and tissue acts as a reservoir for the formation of oestrone by the action of oestrone sulphatase³³.

10 In this regard, it is now believed that the oestrone sulphatase (E1-STS) pathway - i.e. the hydrolysis of oestrone sulphate to oestrone (E1S to E1) is the major source of oestrogen in breast tumours^{1,2}. This theory is supported by a modest reduction of plasma oestrogen concentration in postmenopausal women with breast cancer treated by aromatase inhibitors, such as aminoglutethimide and 4-hydroxyandrostenedione^{3,4}
15 and also by the fact that plasma E1S concentration in these aromatase inhibitor-treated patients remains relatively high. The long half-life of E1S in blood (10-12 h) compared with the unconjugated oestrogens (20 min)⁵ and high levels of steroid sulphatase activity in liver and, normal and malignant breast tissues, also lend support to this theory⁶.

20

Thus, oestrogen formation in malignant breast and endometrial tissues *via* the sulphatase pathway makes a major contribution to the high concentration of oestrogens which are present in these tumours^{24,25}.

25 PCT/GB92/01587 teaches novel steroid sulphatase inhibitors and pharmaceutical compositions containing them for use in the treatment of oestrone dependent tumours, especially breast cancer. These steroid sulphatase inhibitors are sulphamate esters, such as N,N-dimethyl oestrone-3-sulphamate and, preferably, oestrone-3-sulphamate (otherwise known as "EMATE").

30

EMATE is a potent E1-STS inhibitor as it displays more than 99% inhibition of E1-STS activity in intact MCF-7 cells at 0.1 μ M. EMATE also inhibits the E1-STS enzyme in a time-dependent and concentration-dependent manner, thereby indicating that it acts as an active site-directed inactivator^{7,3}.

5

Although EMATE was originally designed for the inhibition of E1-STS, it also inhibits dehydroepiandrosterone sulphatase (DHA-STS), which is an enzyme that is believed to have a pivotal role in regulating the biosynthesis of the oestrogenic steroid androstenediol^{8,9}. This is of significance as there is now evidence to suggest that androstenediol may be of even greater importance as a promoter of breast tumour growth¹⁰.

10

EMATE is also active *in vivo* as almost complete inhibition of rat liver E1-STS (99%) and DHA-STS (99%) activities resulted when it is administered either orally or subcutaneously¹¹. In addition, EMATE has been shown to have a memory enhancing effect in rats¹⁴. Studies in mice have suggested an association between DHA-STS activity and the regulation of part of the immune response. It is thought that this may also occur in humans^{15,16}. The bridging *O*-atom of the sulphamate moiety in EMATE is believed to be important for inhibitory activity. Thus, when the 3-*O*-atom is replaced by other heteroatoms - as in oestrone-3-*N*-sulphamate and oestrone-3-*S*-sulphamate - these analogues are weaker non-time-dependent inactivators¹².

15

20

Thus, EMATE is a potent steroid sulphatase inhibitor which blocks the hydrolysis of both E1S and DHA-S^{29,31}. This inhibitor, therefore, not only blocks the synthesis of oestrone from E1S but also the formation of androstenediol from DHA-S.

25

In addition to oestrone, the other major steroid with oestrogenic properties which is produced by postmenopausal women is androstenediol (see Figure 2).

Androstenediol, although an androgen, can bind to the oestrogen receptor (ER) and can stimulate the growth of ER positive breast cancer cells and the growth of carcinogen-induced mammary tumours in the rat^{26,27}. Importantly, in postmenopausal women 90% of the androstenediol produced originates from the androgen dehydroepiandrosterone sulphate (DHA-S) which is secreted in large amounts by the adrenal cortex. DHA-S is converted to DHA by DHA sulphatase, which may be the same as, or different from, the enzyme, oestrone sulphatase, which is responsible for the hydrolysis of E1S²⁸.

During the last 10-15 years considerable research has also been carried out to develop potent aromatase inhibitors, some of which are currently undergoing clinical evaluation. However, in three recent reports of postmenopausal women with breast cancer who received aromatase inhibitor therapy, plasma E1S concentrations remained between 400-1000 pg/ml³²⁻³⁴.

In summation therefore *in situ* synthesis of oestrogen is thought to make an important contribution to the high levels of oestrogens in tumours and therefore specific inhibitors of oestrogen biosynthesis are of potential value for the treatment of endocrine-dependent tumours.

Moreover, even though oestrogen formation in malignant breast and endometrial tissues *via* the sulphatase pathway makes a major contribution to the high concentration of oestrogens, there are still other enzymatic pathways that contribute to *in vivo* synthesis of oestrogen.

Thus, there is an urgent need to develop new therapies for the treatment of these cancers.

The present invention therefore seeks to overcome one or more of the problems associated with the prior art methods of treating breast and endometrial cancers.

According to a first aspect of the present invention there is provided a sulphamate compound suitable for use as an inhibitor of both oestrone sulphatase activity and aromatase activity.

- 5 In a highly preferred embodiment, the compound of the present invention is a non-steroidal compound.

10 According to a second aspect of the present invention there is provided a compound having the general formula II wherein F represents a phenolic ring structure (a first ring structure), J represents a third ring structure, I represents a phenolic ring structure (a second ring structure), G is an optional double bond, H is a link joining the second ring structure to the third ring structure, and Y represents a suitable second group; wherein any one of ring structures F, J and I has bound thereto a sulphamate group.

15

According to a third aspect of the present invention there is provided a compound according to the present invention for use as a pharmaceutical.

20 According to a fourth aspect of the present invention there is provided a compound according to the present invention for inhibiting oestrone sulphatase activity and aromatase activity.

25 According to a fifth aspect of the present invention there is provided a pharmaceutical composition comprising a compound according to the present invention; and a pharmaceutically acceptable carrier, excipient or diluent.

According to a sixth aspect of the present invention there is provided the use of a compound according to the present invention in the manufacture of a pharmaceutical for inhibiting oestrone sulphatase activity and aromatase activity.

According to a seventh aspect of the present invention there is provided a process for preparing a compound according to the present invention, the process comprising sulphating a flavone, isoflavone or a flavanone.

- 5 According to an eighth aspect of the present invention there is provided a process for preparing a compound according to the present invention, the process comprising sulphamoylating a flavone, isoflavone or a flavanone.

- 10 In one aspect, therefore, the present invention provides a compound, or a pharmaceutical composition comprising the same, that can affect, such as substantially inhibit, not only the oestrone sulphatase pathway - which pathway converts oestrone to and from oestradiol - but also the aromatase pathway - which pathway converts the androgen precursor androstenedione to oestrone.

- 15 This aspect of the present invention is advantageous because by the administration of one type of compound it is possible to block the synthesis of oestrone from both androstenedione and E1S.

- 20 In addition, the present invention is further advantageous in that it may also be possible to block the formation of androstenediol from DHA-S.

Hence, the present invention provides compounds that have considerable therapeutic advantages, particularly for treating breast and endometrial cancers.

- 25 The compounds of the present invention are different from those disclosed in the prior art because they can act as therapeutic agents that possess both aromatase and steroid sulphatase inhibitory properties.

In a preferred embodiment the compound of the present invention comprises a first ring structure and a sulphamoyl group, which first ring structure may be substituted and/or unsaturated.

5 Preferably the first ring structure is a phenolic ring structure, which phenolic ring may be substituted.

Preferably, the compound of the present invention further comprises a second ring structure, which second ring structure may be substituted and/or unsaturated.

10

Preferably the second ring structure is a phenolic ring structure, which phenolic ring may be substituted.

15 Preferably, the compound of the present invention further comprises a third ring structure which is intermediate the first ring structure and the second ring structure, which third ring structure may be substituted and/or unsaturated.

20 In a preferred embodiment, the compound of the present invention is a sulphamate of a flavone, an isoflavone or a flavanone. Alternatively, the compound of the present invention is a sulphamate of a benzoflavone - such as the benzoflavone of Figure 10 wherein R is H or OH (ref. 38). The present invention also covers substituted variants of the sulphamate of the benzoflavone of Figure 10.

25

The present invention will now be described by reference to the Formulae presented in Figures 3-9.

In this regard, it is generally preferred that the compound of the present invention has the general formula I wherein A represents the first ring structure, B represents the third ring structure, D represents the second ring structure, C is an optional double bond, E is a link joining the second ring structure to the third ring structure, X represents a suitable first group, and Y represents a suitable second group; wherein any one of ring structures A, B and D is a phenolic ring; and wherein any one of ring structures A, B and D has bound thereto a sulphamate group.

Each of the ring structures can independently comprise from 3 to 20 atoms in the ring, preferably from 4 to 8 atoms in the ring. Preferably, ring A and ring D comprise 6 atoms in the ring.

A further cyclic group may be linked to ring A or D. This cyclic group may be linked to two spaced-apart atoms in ring A or ring D, such as the structure shown in figure 10.

Preferably, the first ring structure and the second ring structure are substituted.

Preferably, any one of ring structures A and D has bound thereto a sulphamate group.

Preferably, each of the first ring and the second ring is a homogeneous ring structure - i.e. the ring is made up of the same atoms.

Preferably, each of the first ring and the second ring comprises only carbon atoms in the ring.

Preferably, X is C=O.

5 Preferably, the compound of the present invention has the general formula II wherein F represents a phenolic ring structure (the first ring structure), J represents the third ring structure, I represents a phenolic ring structure (the second ring structure), G is an optional double bond, H is a link joining the second ring structure to the third ring structure, and Y represents a suitable second group; wherein any one of ring structures F, J and I has bound thereto a sulphamate group.

10 Preferably, the third ring structure is a heterogeneous ring structure - i.e. different atoms are in the ring.

Preferably, Y is O.

15 Preferably any one of the ring structures F and I has bound thereto a sulphamate group.

20 Preferably, link E or link H is a bond.

25 Preferably, the compound of the present invention is a sulphamate of any one of a flavone, an isoflavone or a flavanone.

Preferably, the compound of the present invention is any one of a compound of the general formula IV, a compound of the general formula V, or a compound of the general formula VI; wherein R_1 - R_{12} are independently selected from H, OH, a halogen, an amine, an amide, a sulphonamine, a sulphonamide, any other sulphur containing group, a saturated or unsaturated C_{1-10} alkyl, an aryl group, a saturated or unsaturated C_{1-10} ether, a saturated or unsaturated C_{1-10} ester, a phosphorus containing group; and wherein at least one of R_1 - R_{12} is a sulphamate group.

Preferably, the sulphamate group has the general formula $OSO_2NR_{13}R_{14}$ wherein R_{13}

and R_{14} are independently selected from H, OH, a halogen, a saturated or unsaturated C_{1-10} alkyl, an aryl group, a saturated or unsaturated C_{1-10} ether, a saturated or unsaturated C_{1-10} ester. Each of R_{13} and R_{14} may be other suitable groups.

- 5 Preferably, the compound of the present invention is any one of a compound of the general formula IV, a compound of the general formula V, or a compound of the general formula VI; wherein R_1 - R_{12} are independently selected from H, OH, $OSO_2NR_{13}R_{14}$, $O-CH_3$; wherein at least one of R_1 - R_{12} is $OSO_2NR_{13}R_{14}$, and wherein R_{13} and R_{14} are defined as above.

10

Preferably, at least one of R_{13} and R_{14} is H. Preferably, each of R_{13} and R_{14} is H.

15

Preferably, the compound of the present invention is a sulphamate of any one of the flavone of formula VII, the isoflavone of formula VIII, or the flavanone of formula IX.

Preferably, the compound of the present invention is the sulphamate of any one of formula VII, formula VIII or formula IX.

20

Preferably, the compound of the present invention is a sulphamate of any one of a flavone, an isoflavone or a flavanone; and wherein the sulphonamoyl group is on the C4' atom of the flavone, isoflavone or flavanone. The C4' position has been shown in general Formula III according to the present invention.

25

Preferably, the compound of the present invention is a flavonoid or flavanoid sulphamate.

In summation the present invention provides compounds that avoid the need for polytherapy. In this regard, the compounds of the present invention can act as

therapeutic agents that possess both aromatase and steroid sulphatase inhibitory properties.

5 Preferably, if the sulphamate group of the compound of the present invention were to be replaced with a sulphate group so as to form a sulphate compound then that sulphate compound would be hydrolysable by an enzyme having steroid sulphatase (E.C. 3.1.6.2) activity.

10 The compound of the present invention may have one or more sulphamate groups. For example, the compound may be a mono-sulphamate or a bis-sulphamate. For example, in Figures 7, 8 and 9 R_1 and R_2 may be each a sulphamate.

Figures 1 and 2 present schematic pathways; Figure 3-10 present chemical formulae; and Figure 11 presents a graph.

15

The present invention will now be described only by way of example.

COMPOUNDS SYNTHESISED

20 The following sulphamate derivatives were synthesised from the following parent compounds:

<u>PARENT COMPOUND</u>	<u>SULPHAMATE COMPOUND</u>
25 1	2
3	4
5	6
7	8
9	10

30

wherein

1 = 6-hydroxy flavone

2 = flavone-6-sulphamate

3 = 7-hydroxy flavone

4 = flavone-7-sulphamate

- 5 = 5,7-dihydroxy flavone
6 = 5-hydroxy-flavone-7-sulphamate
7 = 5,7-dihydroxy-4'-hydroxy-flavone
8 = 5,7-dihydroxy flavanone-4'-flavanone sulphamate
5 9 = 5,7-dihydroxy-4'-methoxy-isoflavone
10 = 5-hydroxy-4'-methoxy-isoflavone-isoflavone-7-sulphamate

The formulae are presented in Figures 7-9.

10 SYNTHESIS

The sulphamate derivatives were prepared essentially as described previously²⁹. In this regard, a solution of the appropriate flavone, isoflavone or flavanone in anhydrous DMF was treated with sodium hydride (60% dispersion; 1 equiv for 2 and
15 4; 2 equiv for 6, 8 and 10) at 0°C under an atmosphere of N₂. After evolution of hydrogen had ceased, sulfamoyl chloride (2 equiv except for 8, 5 equiv) was added and the reaction mixture was poured into brine after warming to room temperature overnight and diluting with ethyl acetate. The organic fraction was washed
20 exhaustively with brine, dried (MgSO₄), filtered and evaporated. The crude product obtained was purified by flash chromatography and recrystallisation to give the corresponding sulfamate.

Flavone 6-O-sulphamate (2)

25 6-Hydroxyflavone (1.0 g, 4.113 mmol) gave crude product (1.21 g) which was fractionated on silica (200 g) with ethyl acetate. Upon evaporation, the first fraction gave a creamy residue (760 mg, 58.2%) which was recrystallised in warm acetone/hexane (3:2) to give 2 as creamy rod-shaped crystals (557 mg), m.p. 190-191°C; R_f = 0.71 (ethyl acetate), 0.51 (ethyl acetate/hexane, 2:1), ν_{max} (KBr)

3260, 3040, 1620, 1600, 1580, 1370, 1180 cm^{-1} ; δ_{H} (acetone- d_6) 6.917 (1H, s, C-3-H), 7.355 (2H, br s, exchanged with D_2O , $-\text{OSO}_2\text{NH}_2$), 7.64 (3H, m, C-3'-H, C-4'-H and C-5'-H), 7.75 (1H, dd, $J_{\text{C-8-H, C-7-H}} = 9 \text{ Hz}$ and $J_{\text{C-5-H, C-7-H}} = 3 \text{ Hz}$, C-7-H), 7.87 (1H, d, $J_{\text{C-7-H, C-8-H}} = 9 \text{ Hz}$, C-8-H), 8.02 (1H, d, $J_{\text{C-7-H, C-5-H}} = 3 \text{ Hz}$, C-5-H) and 8.13 (2H, m, C-2'-H and C-6'-H). MS: m/z (E.I., rel. intensity) 317.0(11), 304.2(6), 238.0(96), 210.0(16), 187.1(14), 152.0(8), 136.0(100). Acc. MS (E.I.): m/z 317.0296, $\text{C}_{15}\text{H}_{11}\text{NO}_5\text{S}$ requires 317.0358. Found C, 56.7; H, 3.44; N, 4.31. $\text{C}_{15}\text{H}_{11}\text{NO}_5\text{S}$ requires C, 56.78; H, 3.49; N, 4.41%.

10 Flavone 7-O-sulphamate (4)

7-Hydroxyflavone (700 mg, 2.938 mmol) gave crude product (770 mg) which was fractionated on silica (200 g) with ethyl acetate. Upon evaporation, the first fraction gave a light brown residue (132 mg) which was recrystallised in hot isopropyl alcohol to give 4 as white needle-shaped crystals (60 mg), m.p. 172-174°C (dec.); $R_{\text{f}} = 0.78$ (ethyl acetate), 0.56 (ethyl acetate/hexane, 4:1); ν_{max} (KBr) 3260, 3100, 1630, 1600, 1400, 1580, 1200, 1150 cm^{-1} ; δ_{H} (DMSO- d_6 /CDCl₃, ca. 1:20) 6.824 (1H, s, C-3-H), 7.396 (1H, dd, $J_{\text{C-5-H, C-6-H}} = 8.8 \text{ Hz}$ and $J_{\text{C-8-H, C-6-H}} = 2.2 \text{ Hz}$, C-6-H), 7.47 (2H, br s, exchanged with D_2O , $-\text{OSO}_2\text{NH}_2$), 7.55 (3H, m, C-3'-H, C-4'-H and C-5'-H), 7.639 (1H, d, $J_{\text{C-6-H, C-8-H}} = 2.2 \text{ Hz}$, C-8-H), 7.92 (2H, m, C-2'-H and C-6'-H) and 8.220 (1H, d, $J_{\text{C-6-H, C-5-H}} = 8.8 \text{ Hz}$, C-5-H). Found: C, 56.5; H, 3.36; N, 4.19. $\text{C}_{15}\text{H}_{11}\text{NO}_5\text{S}$ requires C, 56.78; H, 3.49; N, 4.41%.

25 5-Hydroxyflavone 7-O-Sulphamate (6)

5,7-Dihydroxyflavone (1.0 g, 3.933 mmol) gave crude product (1.13 g) which was fractionated on silica (200 g) with chloroform/acetone (8:1). Upon evaporation, the second fraction gave a yellow residue (324 mg, 24.7%) which was recrystallised in ethyl acetate/hexane (1:1) to give 6 as yellow crystals (213 mg), m.p. 195-200°C (dec.); $R_{\text{f}} = 0.21$, 0.25 and 0.44 for chloroform/acetone 12:1, 8:1 and 4:1

respectively: ν_{\max} (KBr) 3360, 3250, 2925-2850, 1650, 1610, 1380 cm^{-1} , δ_{H} (acetone- d_6) 6.75, 6.98, 7.17 (3H, three s, C-3-H, C-6-H, C-8-H), 7.63 (2H, br s, exchanged with D_2O , - OSO_2NH_2), 7.65 (3H, m, C-3'-H, C-4'-H and C-5'-H), 8.15 (2H, d, $J = 7.7$ Hz, C-2'-H and C-6'-H) and 13.0 (1H, br s, exchanged with D_2O , C-5-OH). MS: m/z (+ve ion FAB in m-NBA, rel. intensity) 440.1(10), 389.3(10), 334.1[100, (M+H) $^+$], 288.1(17), 255.0[25, (M+H-79) $^+$], 169.1(30). MS: m/z (-ve ion FAB in m-NBA, rel. intensity) 499.0(30), 484.1[14, (M-2H+153) $^-$], 475.1(20), 443.1(24), 332.1[100, (M-H) $^-$], 308.1(28), 274.1(20), 253.1[50, (M-H-79) $^-$], 195.1(24). Acc. MS (+ve ion FAB in m-NBA): m/z 334.0392. $\text{C}_{15}\text{H}_{12}\text{NO}_6\text{S}$ requires 334.0385. Found: C, 54.0; H, 3.39; N, 4.21. $\text{C}_{15}\text{H}_{11}\text{NO}_6\text{S}$ requires C, 54.03; H, 3.33; N, 4.20%.

5,7-Dihydroxyflavanone 4'-O-sulphamate (8)

4',5,7-Trihydroxyflavanone (1.0 g, 3.675 mmol) gave crude product (965 mg) which was fractionated on silica (200 g) with ethyl acetate/hexane (4:1) to give a mixture of the starting flavanone and product. This mixture was further fractionated on silica (200 g) with chloroform/acetone (4:1) and upon evaporation, the second fraction gave a pale yellow oil (345 mg, 34%) which solidified on standing. Subsequent recrystallisation of this solid in ethyl acetate/hexane (1:1) gave 8 as white crystals (259 mg), m.p. 211-213°C; $R_f = 0.21$ (chloroform/acetone, 4:1); ν_{\max} (KBr) 3420, 3340, 3260, 3140, 1640, 1510, 1380, 1160 cm^{-1} ; δ_{H} (acetone- d_6) 2.84 (1H, dd, $J_{\text{AB}} = 17.4$ Hz and $J_{\text{ax,eq}} = 3.1$ Hz, C-3-H_B), 3.19 (1H, dd, $J_{\text{BA}} = 16.9$ Hz and $J_{\text{ax,ax}} = 12.8$ Hz, C-3-H_A), 5.62 (1H, dd, $J_{\text{ax,eq}} = 3.1$ Hz and $J_{\text{ax,ax}} = 12.8$ Hz, C-2-H), 5.98 (1H, d, $J = 2.0$ Hz, C-6-H or C-8-H), 6.01 (1H, d, $J = 2.0$ Hz, C-6-H or C-8-H), 7.20 (2H, br s, exchanged with D_2O , - OSO_2NH_2), 7.40 (2H, d, $J = 8.7$ Hz, C-2'-H and C-6'-H), 7.66 (2H, d, $J = 8.7$ Hz, C-3'-H and C-5'-H), 9.65 (1H, br s, C-7-OH) and 12.15 (1H, s, C-5-OH). MS: m/z (+ve ion FAB in m-NBA, rel. intensity) 352.0[100, (M+H) $^+$], 288.1(10), 272.1[14, (M-79) $^+$], 255.2(9), 169.0(13). MS: m/z (-ve ion FAB in m-NBA, rel. intensity) 701.2(12), 606.2(10), 517.1(42), 504.1[20, (M+153) $^-$], 473.2(10), 350.1[100, (M-H) $^-$], 271.1[45, (M-H-79) $^-$], 182.0(8). Acc. MS (+ve ion FAB in m-NBA): m/z 352.0496. $\text{C}_{15}\text{H}_{14}\text{NO}_7\text{S}$ requires 352.0491.

Found: C, 51.1; H, 3.68; N, 3.98. $C_{15}H_{13}NO_7S$ requires C, 51.28; H, 3.73; N, 3.99%.

5-Hydroxy-4'-methoxyisoflavone 7'-O-sulphamate (10)

5 5,7-Dihydroxy-4'-methoxyisoflavone (800 mg, 2.817 mmol) gave crude product (650 mg) which was fractionated on silica (200 g) with chloroform/acetone (8:1). Upon evaporation, the second fraction gave a yellow residue (266 mg, 26%) which was recrystallised in ethyl acetate/hexane (1:1) to give 10 as yellow crystals (211 mg),
10 m.p. 184-188°C; R_f s = 0.22 and 0.59 for chloroform/acetone 8:1 and 4:1 respectively; ν_{\max} (KBr) 3300-3020, 1660, 1610, 1400 cm^{-1} ; δ_H (acetone- d_6) 3.86 (3H, s, $-\text{OCH}_3$), 6.75 (1H, d, $J = 2.2$ Hz, C-6-H or C-8-H), 7.04 (3H, m, C-6-H or C-8-H and C-3'-H and C-5'-H), 7.49 (2H, br s, exchanged with D_2O , $-\text{OSO}_2\text{NH}_2$), 7.58 (2H, d, $J = 7$ Hz, C-2'-H and C-6'-H), 8.41 (1H, s, C-2-H), 13.05 (1H, br s, exchanged with D_2O , C-5-OH). MS: m/z (+ve ion FAB in m-NBA, rel. intensity) 393.3(12), 364.0[100, (M+H) $^+$], 284.1[12, (M-79) $^+$], 169.1(24), 134.0(22). MS: m/z (-ve ion FAB in m-NBA, rel. intensity) 529.1(25), 515.1[12, (M-H+153)], 442.1(20), 362.1[100, (M-H)], 308.1(34), 283.1[70, (M-H-79)], 170.1(26). Acc. MS (+ve ion FAB in m-NBA): m/z 364.0494, $C_{16}H_{14}NO_7S$ requires 364.0491. Found: C, 52.8; H, 3.65; N, 3.81. $C_{16}H_{13}NO_7S$ requires C, 52.89; H, 3.61; N, 3.85%.

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5-Hydroxy Isoflavone-4',7-O,O-Dsulphamate (11) and 5,7-Dihydroxy Isoflavone-4'-O-Sulphamate (12)

4'.5,7-Trihydroxy isoflavone (0.5 g, 1.85 mmol) upon sulphamoylation gave a crude product (0.65 g) which was fractionated on silica (200 g) with chloroform/acetone (4:1), and upon evaporation the third fraction gave a light yellow residue (0.329 g, 51%) which was recrystallized in ethylacetate/hexane (1:2) to give compound (11) as beige crystals (0.197 g); m.p. = > 198°C (dec); R_s = 0.14 and 0.24 for chloroform/acetone 4:1 and 2:1 respectively; ν^{\max} (KBr) 3460 (-NH₂), 1650 (C=O), 1400 (-SO₂N-) cm⁻¹; δ_H (acetone-d₆) 6.78 (1H, d, J = 2.2 Hz, C-6-H or C-8-H), 7.03 (1H, d, J = 2.2 Hz, C-8-H or C-6-H), 7.4 (4H, br s, exchanged with D₂O, C-4'-OSO₂NH₂ and C-7-OSO₂NH₂), 7.43 (2H, d, J = 8.4 Hz, C-3'-H and C-5'-H or C-2'-H and C-6'-H and C-6'-H), 7.72 (2H, d, J = 8.4 Hz, C-2'-H and C-6'-H or C-3'-H and C-5'-H), 8.51 (1H, s, C-2-H) and 12.93 (1H, s, C-5-OH). MS: m/z (+ve ion FAB in m- NBA, rel. intensity) 428.9 [100, (M+H)⁺], 350.0 [20, (M+H-SO₂NH₂)⁺], 272.1 [30, (M-H-SO₂NH₂)⁺]. MS: m/z (-ve ion FAB in m- NBA, rel. intensity) 426.9 [100, (M-H)], 347.9 [95, (M-H-SO₂NH₂)], 269.0 [30, (M-H-SO₂NH₂)]. Acc. MS: m/z (FAB)⁺ 429.0083 C₁₅H₁₃N₂O₉S₂ requires 429.0063. Found C, 42.0; H, 2.91; N, 6.45; C₁₅H₁₂N₂O₉S₂ requires C, 42.06; H, 2.82; N, 6.54%.

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The second fraction was collected and upon evaporation gave light yellow residue (0.112 g, 17%) which was recrystallized in ethylacetate/hexane (1:3) to give compound (12) as pale white crystals (0.068 g); m.p. = 189-192°C R_s = 0.23 and 0.33 for chloroform/acetone 4:1 and 2:1 respectively; ν^{\max} (KBr) 3500-3300 (-NH₂), 3200 (H-bonded-OH), 1680 (C=O), 1610, 1400 (-SO₂N-)cm⁻¹; δ_H (acetone-d₆) 6.32 (1H, d, J = 2.2 Hz, C-6-H or C-8-H), 6.46 (1H, d, J = 2.2 Hz, C-8-H or C-6-H), 7.32 (2H, br s, exchanged with D₂O, -SO₂NH₂), 7.42 (2H, t, J = 8.4 Hz, C-3'-H and C-5'-H or C-2'-H and C-6'-H), 7.69 (2H, d, J = 8.4 Hz, C-2'-H and C-6'-H or C-3'-H and C-5'-H), 8.31 (1H, s, C-2-H), 9.53 (1H, s, C-7-OH) and 12.9 (1H, s, C-5-OH). MS: m/z (+ve ion FAB in m- NBA, rel. intensity) 350.0 [100, (M+H)⁺], 271.1 [15, (M+H-SO₂NH₂)⁺]. MS: m/z (-ve ion FAB in m- NBA, rel. intensity) 347.9 [100, (M-H)], 269.0 [20, (M-H-SO₂NH₂)]. Acc. MS: m/z (FAB)⁻ 350.0347

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$C_{15}H_{12}NO_7S$ requires 350.0335. Found C, 51.0; H, 3.16; N, 3.90; $C_{15}H_{11}NO_7S$ requires C, 51.58; H, 3.17; N, 4.01%.

Isoflavone-4',7-*O,O*-Disulphamate (13)

- 5 4',7-Dihydroxy isoflavone (0.45 g, 1.77 mmol) upon sulphamoylation gave a crude product (0.769 g) which was fractionated on silica (200 g) with chloroform/acetone (4:1), and upon evaporation the second fraction gave a pale white residue (0.553 g, 72%) which was recrystallized in acetone/hexane (1:2) to give the compound (13) as white crystals (0.327 g); m.p. > 195°C (dec.); R_f s = 0.21 and 0.40 for
- 10 chloroform/acetone 4:1 and 2:1 respectively; ν^{max} (KBr) 3400 ($-NH_2$), 1640 (C=O), 1360 ($-SO_2N-$) cm^{-1} . δ_H (DMSO- d_6), 7.37 (2H, d, $J = 8.8$ Hz, C-3'-H and C-5'-H or C-2'-H and C-6'-H), 7.42 (1H, dd, $J_{C-6-H, C-8-H} = 2.2$ Hz, $J_{C-6-H, C-5-H} = 8.8$ Hz, C-6'-H), 7.7 (2H, d, $J = 8.8$ Hz, C-2'-H and C-6'-H or C-3'-H and C-5'-H), 8.09 (2H, br s, exchanged with D_2O , $-OSO_2NH_2$), 8.24 (1H, d, $J = 8.8$ Hz, C-5'-H), 8.36
- 15 (2H, br s, exchanged with D_2O , $-OSO_2NH_2$), 8.63 (1H, s, C-2'-H). MS: m/z (+ve ion FAB in m- NBA, rel. intensity) 412.9 [100, (M+H) $^+$], 334.0 [25, (M+H- SO_2NH_2) $^+$], 255.1 [20, (M+H- SO_2NH_2) $^+$]. MS: m/z (-ve ion FAB in m- NBA, rel. intensity) 410.9 [100, (M-H) $^-$], 332.0 (70, (M-H- SO_2NH_2) $^-$], 253.0 [30, (M-H- SO_2NH_2) $^-$]. Acc. MS: m/z (FAB) $^+$ 413.0119 $C_{15}H_{13}N_2O_8S_2$ requires 413.0113.
- 20 Found C, 44.0; H, 2.94; N, 6.62; $C_{15}H_{12}N_2O_8S_2$ requires C, 43.69; H, 2.93; N, 6.79%.

ASSAY OF INHIBITION OF SULPHATASE AND AROMATASE ACTIVITIES

- 25 Sulphatase inhibition was assessed using placental microsome (100,000 g) preparations or intact MCF-7 breast cancer cells as described previously^{29,30}. Placental microsomes were incubated with 3H EIS, adjusted to 20 μM with unlabelled substrate, in the absence or presence of inhibitor.
- 30 Placental microsomes were also used to assess the aromatase inhibitory properties of the flavanoid sulphamates using a tritiated water release assay³⁷. Further placental

microsomes (200 μ l) were incubated with [1β - 3 H] androstenedione, 60 nM and 1 mM NADPH in the absence or presence of inhibitor.

INHIBITION OF SULPHATASE AND AROMATASE ACTIVITIES

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Inhibition of oestrone sulphatase and aromatase activities in placental microsomes by the flavanoid sulphamate derivatives is shown in the Table below.

COMPOUND	CONCENTRATION	% INHIBITION	% INHIBITION
	μ M	Sulphatase	Aromatase
Flavone-6-sulphamate	1	26.8	1
	10	89.5	6.5
Flavone-7-sulphamate	1	-	55
	10	-	86
	50	56.3	
	100	75.3	
5-hydroxy flavone-7-sulphamate	1	8	5
	10	21	76
5,7-dihydroxy flavanone 4'-sulphamate	0.1	30.4	Not tested
	1	79.1	Not tested
	10	98.1	Not tested
5-hydroxy-4'-methoxy-isoflavone-7-sulphamate	1	1	2
	10	50.6	5

From the results, it can be seen that potent inhibition of sulphatase and aromatase activities was detected. For sulphatase inhibition this ranged from 21% at 10 μ M by 5-hydroxy flavone-7-sulphamate, to 98% by 5,7-dihydroxy flavanone-4'-sulphamate

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at 10 μ M. Potent aromatase inhibition was also achieved ranging from 6.5% by flavone-6-sulphamate at 10 μ M to 86% by flavone-7-sulphamate at 10 μ M.

FURTHER *IN VITRO* TESTING

The following Table presents *in vitro* data for three isoflavones that were tested.

IN VITRO ACTIVITY

Compound	Concentration (μ M)	% Inhibition	
		MCF-7 Cells	Placental Microsomes
Isoflavone 5-hydroxy- 4',7-bissulphamate	0.1	28	nd
	1.0	90	nd
	10.0	99	93
Isoflavone 5,7-dihydroxy- 4'-sulphamate	0.1	23	nd
	1.0	83	nd
	10.0	99	75
Isoflavone-4',7- bissulphamate	0.1	89	nd
	1.0	99	nd
	10.0	99	99

nd = not done

IN VIVO TESTING

Figure 11 presents *in vivo* inhibition of oestrone sulphatase activity in rat liver for two isoflavones according to the present invention. In this regard, BH22F1 = 5-hydroxy isoflavone-4',7-bissulphamate; BH22BF1 = 5,7-dihydroxy isoflavone-4'-sulphamate. Compounds were administered as a single 10 mg/Kg dose. Oestrone sulphatase activity was assayed in tissue samples obtained 24 h after drug administration.

Other modifications of the present invention will be apparent to those skilled in the art.

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CLAIMS

1. A sulphamate compound suitable for use as an inhibitor of both oestrone sulphatase activity and aromatase activity.
- 5 2. A compound according to claim 1 wherein the compound comprises a first ring structure and a sulphamoyl group, which first ring structure may be substituted and/or unsaturated.
- 10 3. A compound according to claim 2 wherein the first ring structure is a phenolic ring structure, which phenolic ring may be substituted.
- 15 4. A compound according to any one of claims 1 to 3 wherein the compound further comprises a second ring structure, which second ring structure may be substituted and/or unsaturated.
5. A compound according to claim 4 wherein the second ring structure is a phenolic ring structure, which phenolic ring may be substituted.
- 20 6. A compound according to any one of claims 1 to 5 wherein the compound further comprises a third ring structure which is intermediate the first ring structure and the second ring structure, which third ring structure may be substituted and/or unsaturated.
- 25 7. A compound according to claim 6 wherein the compound has the general formula I: wherein A represents the first ring structure; B represents the third ring structure, D represents the second ring structure, C is an optional double bond, E is a link joining the second ring structure to the third ring structure, X represents a suitable first group, and Y represents a suitable second group; wherein any one of ring

structures A, B and D is a phenolic ring; and wherein any one of ring structures A, B and D has bound thereto a sulphamate group.

5 8. A compound according to claim 7 wherein the compound has the general formula II wherein F represents a phenolic ring structure (the first ring structure), J represents the third ring structure, I represents a phenolic ring structure (the second ring structure), G is an optional double bond, H is a link joining the second ring structure to the third ring structure, and Y represents a suitable second group; wherein any one of ring structures F, J and I has bound thereto a sulphamate group.

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9. A compound having the general formula II wherein F represents a phenolic ring structure (a first ring structure), J represents a third ring structure, I represents a phenolic ring structure (a second ring structure), G is an optional double bond, H is a link joining the second ring structure to the third ring structure, and Y represents
15 a suitable second group; wherein any one of ring structures F, J and I has bound thereto a sulphamate group.

10. A compound according to any one of claims 7 to 9 wherein the first ring structure and the second ring structure are substituted.

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11. A compound according to any one of claims 7 to 10 wherein any one of ring structures A or F and D or I has bound thereto a sulphamate group.

12. A compound according to any one of claims 7 to 11 wherein the third ring structure is a heterogeneous ring structure.
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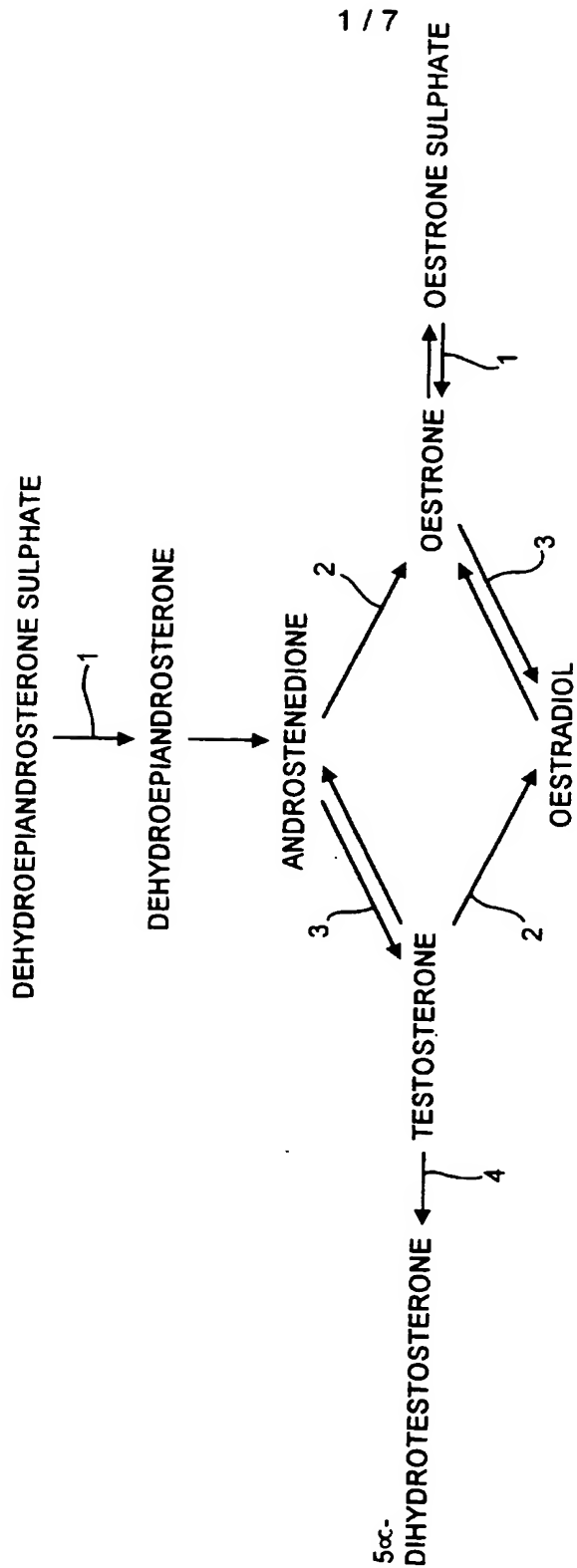
13. A compound according to any one of claims 7 to 12 wherein X is C=O.

14. A compound according to any one of claims 7 to 13 wherein Y is O.

15. A compound according to any one of the preceding claims wherein the compound of the present invention is a sulphamate of any one of a flavone, an isoflavone or a flavanone.
- 5 16. A compound according to claim 15 wherein the compound is any one of: a compound of the general formula IV, a compound of the general formula V or a compound of the general formula VI; wherein R_1 - R_{12} are independently selected from H, OH, a halogen, an amine, an amide, a sulphonamine, a sulphonamide, any other sulphur containing group, a saturated or unsaturated C_{1-10} alkyl, an aryl group, a
10 saturated or unsaturated C_{1-10} ether, a saturated or unsaturated C_{1-10} ester, a phosphorus containing group; and wherein at least one of R_1 - R_{12} is a sulphamate group.
- 15 17. A compound according to any one of the preceding claims wherein the sulphamate group has the general formula $OSO_2NR_{13}R_{14}$ wherein R_{13} and R_{14} are independently selected from H, OH, a halogen, a saturated or unsaturated C_{1-10} alkyl, an aryl group, a saturated or unsaturated C_{1-10} ether, a saturated or unsaturated C_{1-10} ester.
- 20 18. A compound according to any one of the preceding claims wherein the compound is any one of a compound of the general formula IV, a compound of the general formula V or a compound of the general formula VI; wherein R_1 - R_{12} are independently selected from H, OH, $OSO_2NR_{13}R_{14}$, $O-CH_3$; wherein at least one of R_1 - R_{12} is $OSO_2NR_{13}R_{14}$, and wherein R_{13} and R_{14} are defined in claim 17.
- 25 19. A compound according to claim 16 or claim 17 wherein R_{13} and R_{14} are H.
20. A compound according to any one of the preceding claims wherein the compound is a sulphamate of any one of the flavone of formula VII, the isoflavone
30 of formula VIII or the flavanone of formula IX.

21. A compound according to any one of the preceding claims wherein the compound is the sulphamate of any one of formula VII, formula VIII or formula IX.
- 5 22. A compound according to any one of the preceding claims wherein the compound is a sulphamate of any one of a flavone, an isoflavone or a flavanone; and wherein the sulphonamoyl group is on the C4' atom of the flavone, isoflavone or flavanone.
- 10 23. A compound according to any one of the preceding claims wherein the compound is a flavanoid sulphamate.
24. A compound according to any one of the preceding claims for use as a pharmaceutical.
- 15 25. A compound according to any one of claims 1 to 23 for inhibiting oestrogen sulphonatase activity and aromatase activity.
- 20 26. A pharmaceutical composition comprising a compound according to any one of claims 1 to 23; and a pharmaceutically acceptable carrier, excipient or diluent.
27. Use of a compound according to any one of claims 1 to 23 in the manufacture of a pharmaceutical for inhibiting oestrogen sulphonatase activity and aromatase activity.
- 25 28. A process for preparing a compound according to any one of claims 1 to 23, the process comprising sulphonating a flavone, isoflavone or a flavanone.
29. A process for preparing a compound according to any one of claims 1 to 23, the process comprising sulphonamoylating a flavone, isoflavone or a flavanone.

- 30. A compound substantially as described herein.
- 31. A process of preparing a compound substantially as described herein.



KEY ENZYMES IN STEROIDOGENESIS:-

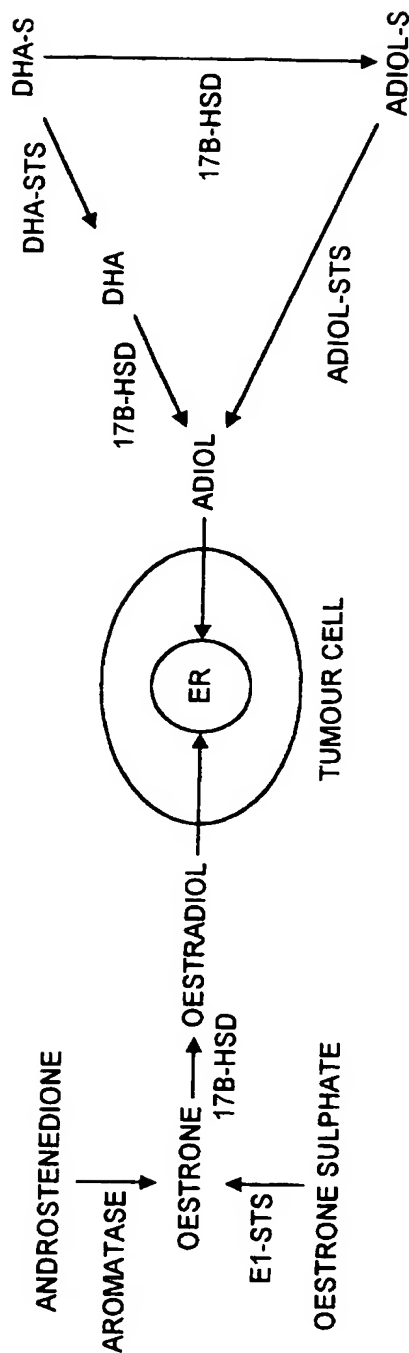
1. SULPHATASE 2. AROMATASE 3. DEHYDROGENASE 4. 5 α REDUCTASE

FIG. 1

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FIG. 2

ORIGIN OF OESTROGENIC STEROIDS IN POSTMENOPAUSAL WOMEN



ER=OESTROGEN RECEPTOR, DHA / -S=DEHYDROEPIANDROSTERONE / -SULPHATE,
 ADIOL=ANDROSTENEDIOL, E1-STS=OESTRONE SULPHATASE, DHA -STS=
 DHA-SULPHATASE, ADIOL-STS=ADIOL SULPHATASE, 17β-HSD=OESTRADIOL 17β-
 HYDROXYSTEROID DEHYDROGENASE

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FIG. 3a

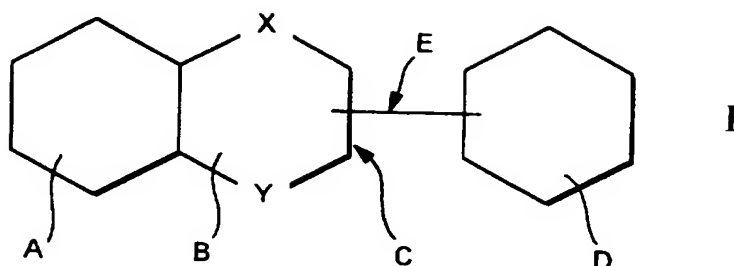


FIG. 3b

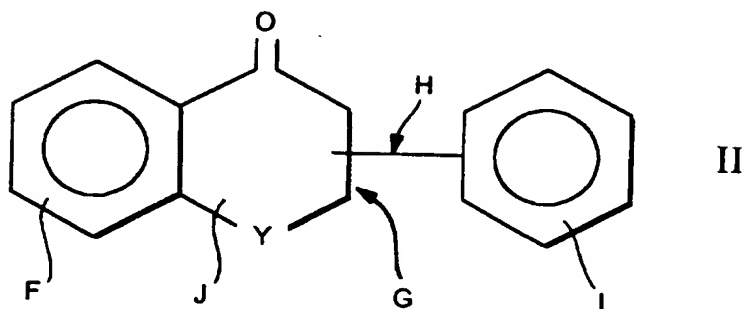
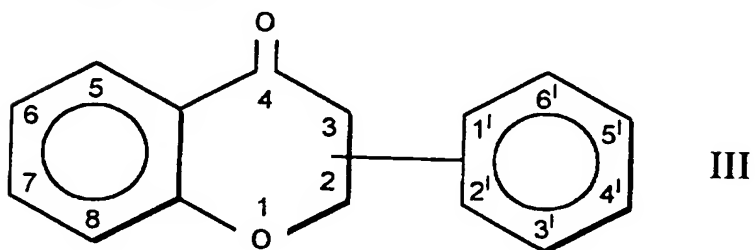
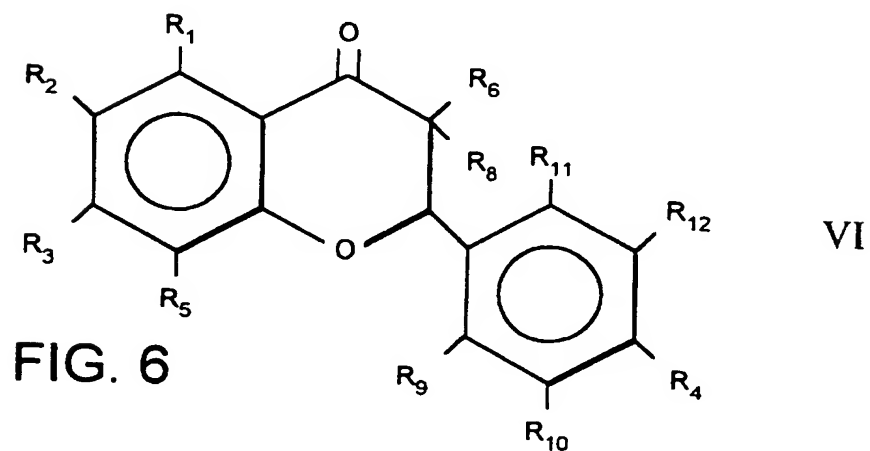
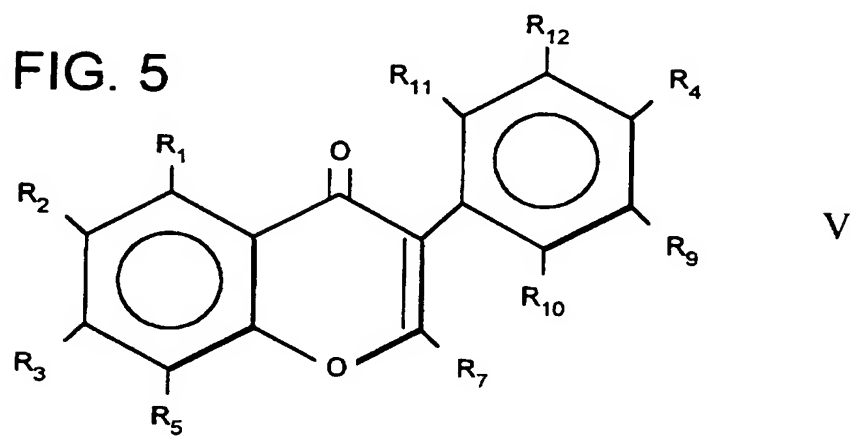
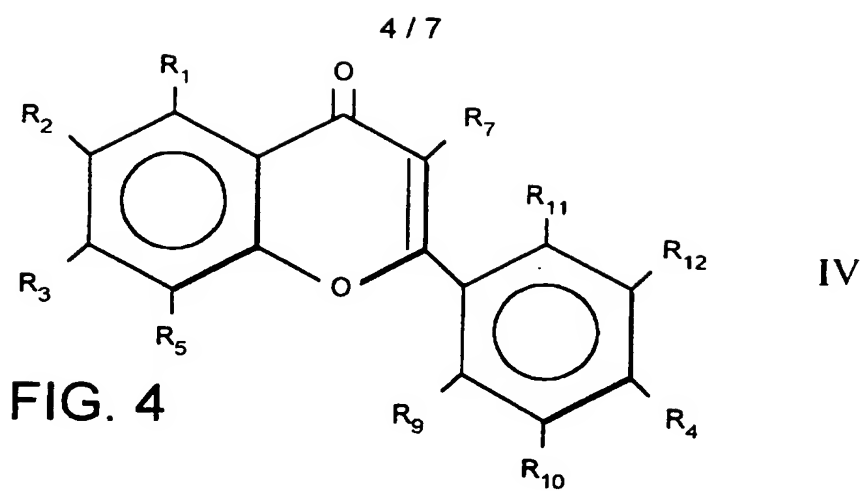


FIG. 3c



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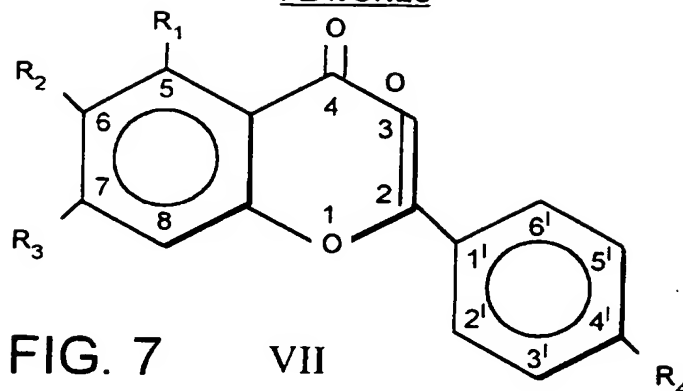
FLAVONES

FIG. 7 VII

	<u>R₁</u>	<u>R₂</u>	<u>R₃</u>	<u>R₄</u>
1	H	OH	H	H
2	H	OSO ₂ NH ₂	H	H
3	H	H	OH	H
4	H	H	OSO ₂ NH ₂	H
5	OH	H	OH	H
6	OH	H	OSO ₂ NH ₂	H

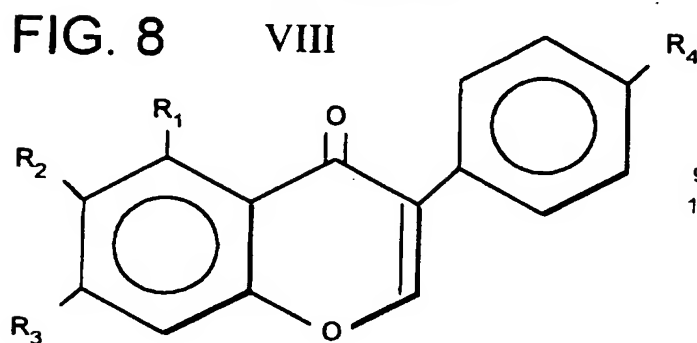
ISOFLAVONES

FIG. 8 VIII

	<u>R₁</u>	<u>R₂</u>	<u>R₃</u>	<u>R₄</u>
9	OH	OH	H	OCH ₃
10	OH	H	OSO ₂ NH ₂	OCH ₃

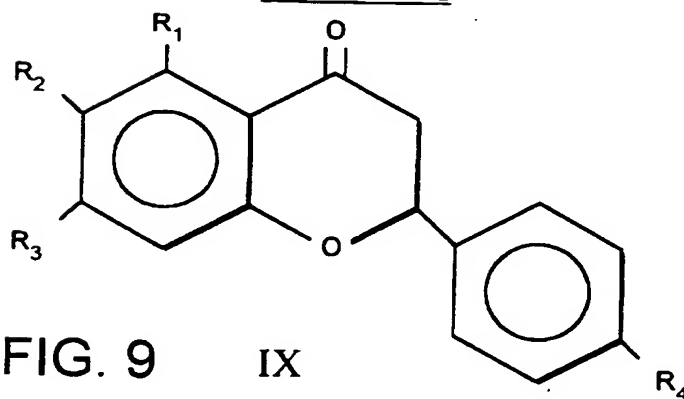
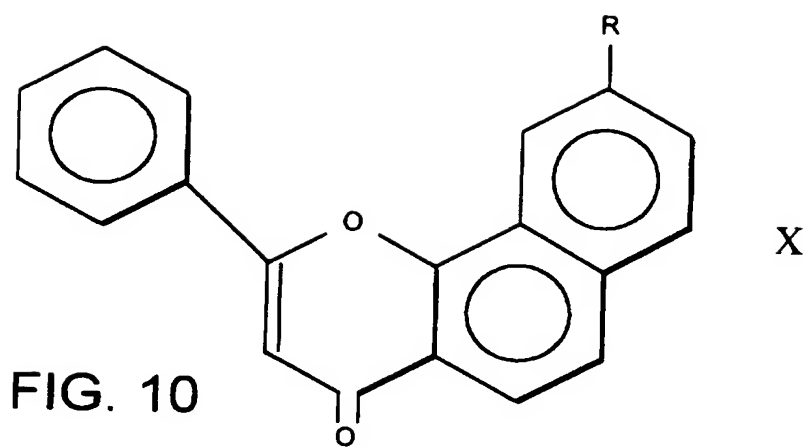
FLAVANONES

FIG. 9 IX

	<u>R₁</u>	<u>R₂</u>	<u>R₃</u>	<u>R₄</u>
7	OH	H	OH	OH
8	OH	H	OH	OSO ₂ NH ₂

SUBSTITUTE SHEET (RULE 26)



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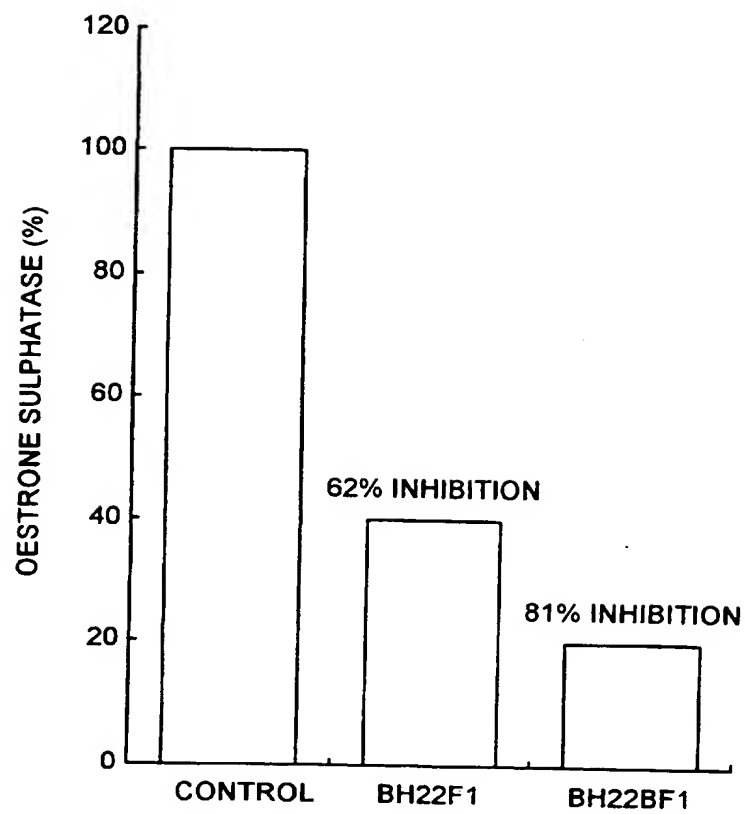


FIG. 11

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 97/00600

A. CLASSIFICATION OF SUBJECT MATTER IPC 6 C07D311/30 C07D311/32 C07D311/36 A61K31/35				
According to International Patent Classification (IPC) or to both national classification and IPC				
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 6 C07D				
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched				
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)				
C. DOCUMENTS CONSIDERED TO BE RELEVANT				
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.		
A	FR 2 543 140 A (CORTIAL) 28 September 1984 see the whole document -----	1,15		
<input type="checkbox"/> Further documents are listed in the continuation of box C.				
<input checked="" type="checkbox"/> Patent family members are listed in annex.				
* Special categories of cited documents :				
<table border="0"> <tr> <td style="vertical-align: top;"> "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed </td> <td style="vertical-align: top;"> "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "A" document member of the same patent family </td> </tr> </table>			"A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "A" document member of the same patent family
"A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "A" document member of the same patent family			
Date of the actual completion of the international search 2 June 1997		Date of mailing of the international search report 11.06.97		
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (- 31-70) 340-2040, Tx. 31 651 epo nl, Fax (- 31-70) 340-3016		Authorized officer Francois, J		

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB 97/00600

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 1-14
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
ART.6 PCT : the definition of claim 1 and followings is very general and encompasses a very broad number of chemical compounds. Thus for economical reasons the search has been limited to benzopyran derivatives with at least one sulfamate radical (Guidelines part B chap.III ; 2.1 ; 3).
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/GB 97/00600

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		US 4600788 A	15-07-86
